CALCIUM

o-CRESOLPHTHALEIN METHOD

For "in vitro" determination of calcium in serum, plasma or urine

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PRINCIPLE

At alkaline pH values, serum calcium forms a coloured complex with o-cresolphthalein, 8-hydroxyquinolein is included in the reagent as a chelating agent of magnesium ions which can interfere with the reaction.

DIAGNOSTIC USE

Calcium is the most abundant cation in the human body (99%) and is distributed mainly in the bones.
High calcium levels are found in the primary and tertiary hyperparathyroidism, cancer diseases (metastatic breast carcinoma, kidney or lung, leukemia, multiple myeloma), vitamin D intoxication, increased renal retention, sarcoidosis, osteoporosis, thyrotoxicosis.
Low calcium levels can be attributed to renal failure, hypoparathyroidism, vitamin D deficiency, malnutrition or malabsorption.

Single test result could not be used to make a clinical diagnosis. It should integrate clinical and laboratory data.

REAGENTS

Kit 2 x 100 mL. (Ref. 99 59 36). Contents:

A. 1 x 100 mL o-Cresolphthalein	Ref. 99 99 36
B. 1 x 100 mL Colour developer	Ref. 99 47 42
C. 1 x 5 mL Standard.	Ref. 99 19 21

WORKING REAGENT PREPARATION

Mix equal volumes of each reagent (A and B).
Standard is ready to use.

WORKING REAGENT COMPOSITION

The reagent composition is as follows:

2-amino-2-methyl-1-propanol	0.35 M
o-cresolphthalein	0.04 mM
8-hydroxyquinolein	12 mM
HCl	2.5 mM
Preservatives and stabilizers	

Standard: Aqueous solution of calcium equivalent to 10 mg/dL (2.49 mmol/L).

STORAGE AND STABILITY

The components of the kit, when stored at 2 - 8°C, will remain stable until the expiration date stated on the label.
The working reagent will remain stable 3 days at room temperature (≤ 25°C) and 15 days at 2-8°C

Signs of reagent deterioration:
Presence of particles or turbidity in the reagent. Working reagent blank >0.300.

ADDITIONAL EQUIPMENT

General laboratory equipment.
Spectrophotometer, photometer or automated analyzer.

SAMPLE

Serum or plasma with heparin or urine. Serum calcium remains unchanged for 10 days at 2-8°C.
It is advisable acidifying the samples of 24 hr urine to avoid precipitation of calcium and dilute with deionized water (1+1) prior to the determination. Multiply the final result by 2.

CAUTION

The safety statements are on the label. Handle the reagent with care.
It is advisable to look at the MSDS before using the reagent.
The disposal of the residues has to be made according to legal local regulations.

QUALITY CONTROL

Control serum, Seriscann Normal (Ref. 99 41 48) and Seriscann Abnormal (Ref. 99 46 85) should be included in each test series. Each particular laboratory should establish its own control program.

AUTOANALYZERS

Adaptations to different autoanalyzers are available on request.

PROCEDURE

Technique	BL mL	ST mL	SA mL
Sample	--	--	0.01
Standard	--	0.01	--
Working reagent	1.00	1.00	1.00

Mix well and incubate at room temperature (20-25°C) for 5 min.

Reading
Wavelength: 565 nm
Blank: BL contents
Colour stability: a minimum of 1 hour

CALCULATIONS

$$\frac{\text{SA Abs}}{\text{ST Abs}} \times 10 = \text{mg de calcium / dL}$$

$$\frac{\text{SA Abs}}{\text{ST Abs}} \times 10 \times 2 \times \text{vol(dL) urine/24h} = \text{mg de calcio / 24h (urine)}$$

Where:
SA Abs: Sample Absorbance
ST Abs: Standard Absorbance

SI Units
(mg/dL) x 0.2495 = mmol/L

REFERENCE VALUES

Serum
Newborn: 8.0 – 13.0 mg/dL
Children: 8.8 – 12.0 mg/dL
Adults: 8.8 – 10.5 mg/dL
Urine: 100 - 300 mg/24 h

The stated values are for guidance. Each particular laboratory should establish its own normal range, using its own instrumentation, blood collection methods and test procedures

PERFORMANCE CHARACTERISTICS

The performance characteristics depend on the method used. It is recommended to calculate these data for each particular test protocol. These results have been obtained using a manual method.

Sensitivity, as detection limit: 0.9 mg/dL
Linearity:20 mg/dL (4.99 mmol/L).Samples that give higher concentration should be diluted in deionised water and the final result has to be multiplied by the dilution factor.
Accuracy: 96.8%
Repetitivity, as CV%: 2.44%
Reproducibility, as CV%: 2.72%
Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagent.

Details of the performance studies are available on request.

INTERFERENCES

Bilirubin concentrations higher than 20 mg/dL, and phosphates higher than 40 mg/dL, will interfere with the assay.
Magnesium ions up to tenfold their normal upper limit in blood will not disturb the final results.
Those anticoagulants which can behave as chelating agents shall never be used with this test (EDTA, fluoride, oxalate...).
To avoid undesirable contaminations it is expressly recommended the use of disposal plasticware.

REFERENCES

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